Form PTO-1390 (Rev. 5-93) US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

Rec'd PCT/PTO 28 OCT 1997

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NO. H 1920 PCT/US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 08/945574

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP96/01755	**April 26, 1995**	**April 28, 1995** **March 12, 1996**

TITLE OF INVENTION

DETERGENTS COMPRISING CELLULASES

APPLICANT(S) FOR DO/EO/US

Hermanus Bernardus Maria Lenting, Rudolf Franciscus Wilhelmus Cornelis Van Beckhoven, Karl-Heinz Maurer, Beatrix Kottwitz, Albrecht Weiss and Pieter Van Solingen

Applicant herewith submits to the United States Designated/Elected Office (EO/DO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. □ This a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. □ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S. C. 371(c)(2)).
   a. □ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. □ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. □ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. □ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. □ have been transmitted by the International Bureau.
   c. □ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. □ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). **(Unexecuted)**
10. □ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. □ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. □ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment
    □ A SECOND or SUBSEQUENT preliminary amendment.
14. □ A substitute specification.
15. □ A change of power of attorney and/or address letter.
16. □ Other items or information.:

"Express Mail" mailing label number TB81136340XUS

Date of Deposit October 28, 1997

U.S. Application No. (If known see CFR1.30)	INTERNATIONAL APPLICATION NO. **PCT/EP96/01755**	ATTORNEY'S DOCKET NUMBER **H 1920 PCT/US**

17. ■ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

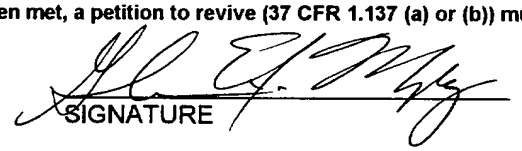
		CALCULATIONS ONLY	PTO USE
Search Report has been prepared by the EPO or JPO.....	**$930.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482)	**$720.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37CFR 1.445(a)(2)).....	**$790.00**		
Neither international preliminary examination fee (37CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....	**$1070.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....	**$98.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT	=	$ 930	00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date 37 (CFR 1.492(e)).		$ 0	00

Claims	Number filed	Number Extra		Rate		
Total Claims	1 - 20 =	0	0	X $22.00	$ 0	00
Independent Claims	1 - 3 =	0	0	X $82.00	$ 0	00
Multiple dependent claims (s)(if applicable)		0		+ $270.00	$ 0	00
TOTAL OF ABOVE CALCULATIONS				=	$ 930	00
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					$ 0	00
SUBTOTAL				=	$ 930	00
Processing fee of **$130.00** for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37CFR 1.492(f)).				+	$ 0	00
TOTAL NATIONAL FEE				=	$ 930	00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property				+	$ 0	00
TOTAL FEES ENCLOSED				=	$ 930	00
					Amount to be: refunded	$----------
					charged	$930.00

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.
b. ■ Please charge my Deposit Account No. 01-1250 in the amount of $930.00 to cover the above fees.
A triplicate copy of this sheet is enclosed. Order No. 97-1230.
c. ■ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-1250. A triplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Henkel Corporation, Law Dept.
140 Germantown Pike, Suite 150
Plymouth Meeting, PA 19462


SIGNATURE

Glenn E. J. Murphy
NAME ATTORNEY FOR APPLICANT
33,539
REGISTRATION NUMBER

Form PTO 1390 (REV 5-93)

#4

27 FEB 1998

"Express Mail" mailing label number TB 811364664 US
Date of Deposit FEB. 27, 1998

08/945574

PATENT
Docket No. H 1920 PCT/US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of Lenting et al.

Serial No. 08/945,574 Examiner: Unknown
Filed: October 28, 1997 Art Unit: Unknown
PCT/EP96/01755
International Filing Date: 26 April 1996
Priority Date Claimed: 28 April 1995 and 12 March 1996
TITLE DETERGENTS COMPRISING CELLULASES

TRANSMITTAL OF DECLARATION
UNDER 37 CFR SECTION 1.494/5(c)

Assistant Commissioner of Patents
Box PCT
Washington, D.C. 20231

Attn: DO/EO/US

Sir:

No original declaration or oath was filed earlier herein. Accordingly, enclosed is the original declaration or oath for this application.

Please charge our Deposit Account No. 01-1250 in the amount of $130.00 as prescribed by 37 CFR 1.492(e) for the surcharge and processing fee for filing a declaration on a date later than 20/30 months after the priority date of the application. This page is being submitted in triplicate along with an executed declaration. Order No. 98-0237. Authorization is also granted to charge any deficiency to Deposit Account 01-1250.

Respectfully submitted,

03/10/1998 PVULPE 00000096 DA#:011250 08945574
01 FC:154 130.00 CH

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PATENT
Docket No. H 1920 PCT/US

"Express Mail" mailing label number TB8113634OXUS
Date of Deposit October 28, 1997

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE: PCT/EP96/01755
International Filing Date: 26 April 1996
Priority Date Claimed: 28 April 1995;
12 March 1996
Applicant: Lenting et al.
Title: **DETERGENTS COMPRISING CELLULASES**
Applicants' Reference: H 1920 PCT/US

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Box PCT
Washington, DC 20231

ATTN: DO/EO/US

Dear Sir:

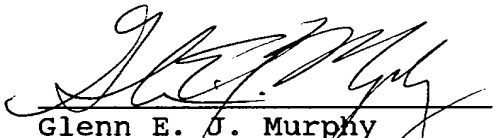
Before examining this application, please enter the amendments below:

IN THE CLAIMS:

Please cancel claims 2 through 13.

Applicants respectfully request entry of this amendment. If any fees are due in connection with entering this amendment, the Commissioner hereby is authorized to charge such fees to our Deposit Account No. 01-1250.

Respectfully submitted,



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DETERGENTS COMPRISING CELLULASES

The present invention relates to the use of novel cellulases with improved properties in detergents and aqueous laundry solutions. The invention further relates to detergents and detergent additives comprising the novel cellulase.

Cellulases, also called cellulolytic enzymes, are enzymes which are capable of the hydrolysis of the ß-D-glucosidic linkages in celluloses. Cellulolytic enzymes have been divided traditionally into three classes: endoglucanases, exoglucanases or cellobiohydrolases and ß-glucosidases (Knowles, J. et al. (1987), TIBTECH 5, 255-261). Cellulolytic enzymes can be produced by a large number of bacteria, yeasts and fungi. Microorganisms that produce cellulases are described in for example GB-A-2094826.

Several applications have been developed for the use of cellulolytic enzymes:

- degrading (wood)cellulose pulp into sugars for (bio)ethanol production;
- several textile treatments like 'stone washing' and 'biopolishing';
- application in detergent compositions.

The use of cellulases in detergent compositions started with cellulases capable of reducing the harshness, i.e. softening, of cotton containing fabrics, as described in for example GB-B-1358599.

It is further known that detergent compositions comprising cellulases are effective in removing dirt, i.e. cleaning. The efficiency of cellulolytic enzymes, cellulases, in terms of cleaning textile has been recognized for some time. GB-A-2075028, GB-A-2095275 and GB-A-2094826 disclose detergent compositions with cellulase for improved cleaning performance.

It is also known in the art that cellulases can act as a colour clarifying agent in laundry detergents. After repeated washing of soiled fabrics, cotton containing fabrics appear to be greyish, most probably due to disrupted fibres caused by mechanical action. The fibres are torn up resulting in disordered fibres which are broken. The use of cellulases as colour clarification agents for coloured fabrics has been described in EP-A-0220016. Actually cellulase mixtures from the fungal strain Humicola insolens (DSM 1800) are

commonly used in detergents to result in antipilling and colour revival properties. The cellulolytic enzyme system produced by the wild type microorganism is available under the trade name of Celluzyme® by Novo-Nordisk. In addition a cloned (single) cellulase from the same origin under the trade name Carezyme® is also used in detergents.

The main disadvantage of the cellulases known in the art showing colour clarification is that these enzymes agressively degrade the cellulose containing fabrics which results in damage by undesirable loss of tensile strength of the fabrics.

On the other hand cellulases known in the art showing good cleaning properties show hardly any colour clarification effects. The first commercial detergent with cellulases in the world contained a bacterial cellulase. This enzyme represents an above mentioned alkaline endoglucanase from a Bacillus species that does not attack cellulose fibers. The enzyme is described to give a cleaning effect during washing. No effects with respect to anti-pilling or colour revival have been described for this enzyme.

From the above it will become clear that it is still desirable to provide for improved cellulases in detergent applications. Using mixtures of cellulases, as suggested in international patent application WO-A-95/02675, is supposed to provide the above mentioned performances in laundry washing, but to our knowledge it has not previously been possible to use single enzymes providing all these characteristics when applied in laundry washing.

Surprisingly it has been found that the use of certain single cellulases which are capable of cleaning, antiredeposition, colour clarification and antipilling performance in laundry washing does not at all result in unacceptable damage to the textiles washed.

Accordingly, the present invention relates to the use of a single cellulase with a ratio of tensile strength loss (TSL, as herein definded) to antipilling properties (AP, as herein defined) below 1 in aqueous laundry solutions.

To measure tensile strength loss is a way to measure damage caused by mechanical stress or enzymatical action on fibers. It is to be understood that for the purpose of the present invention cotton fiber has to be used. The method measures the tensile

strength of single fibers under wet conditions. It is described in the German Standard DIN 53, 857, part 1, as well as in the International Standard ISO 2267.

As the effect normally shows up in a significant amount only after about 20 to 25 wash cycles, there is always some tensile strength loss due to the mechanical forces acting on the cotton fiber during the washing process. Therefore the tensile strength loss of a control fabric washed without cellulases using the same formulation of detergent and the same type of washing machine and washing programme has to be subtracted. To calibrate the values, a preparation of the (single) endoglucanase V from Humicola insolens (EG V) in equal amounts of enzymatic protein in the detergent is used as a standard and the value of the tensile strength loss for this sample minus the control value of detergent without cellulase is taken as a TSL of 100%. This cellulase EG V has been described for example in the international patent application WO 91/17243. The amount of protein can be measured for example by using the BCA Pierce method as described by R.E. Brown et al. in Anal. Biochem. 1989, vol. 180, p. 136 - 139.

A preparation of an above mentioned Bacillus cellulase available from Kao Corp. under the trade mark KAC® 500 or KAC® 700 may be used as comparison, resulting in general in a very low tensile strength loss as compared to the control washing experiment with no cellulase present.

The attack of cellulases on protruding microfibrils, pills and cotton fluff on the surface of a cotton fabric results in an optically visible removal of that pills. To test the effect, washings are to be performed using a detergent with and without cellulase, as described for the detremination of TSL. The antipilling effect, too, can best be seen after an increasing number of wash cycles. Therefore a number of 15 to 40 wash cycles are generally used to demonstrate this effect of cellulases.

There are three different methods that can be used for quantification of this effect:

1. visual evaluation by a test group (panel)
2. measurement of light reflection (L-value of the CIELAB-system)
3. determination of the cotton fluffs by means of optical measurement

The determination using the L-value of the CIELAB-system [Commission Internationale de l'Éclairage] was described by U. Hotz in Tenside Surf. Det. 1993, vol. 30, page 388.

The optical measurement system, which is used in the preferred method of determining the antpilling properties, usually consists of a light source, a microscope tube and a CCD colour camera recording the light reflected from the surface of a fabric. Depending on the amount of pills and fluff on the surface of the fabric the amount of reflected light as measured by digital image analysis changes. Such a system can be used to measure quantitatvely the amount of pill and fluff on fabrics, normally after 15 to 40 wash cycles depending on the type and activity of the cellulase added to the detergent. An optical system which can be used to measure the degree of pilling has been described by T. Müller-Kirschbaum and H. Grundmann in SÖFW, vol. 118 (1992), p. 483-499.

Whatever method is used to determine the antipilling effect of the cellulase to be tested, the standard cellulase EG V has to be tested under the same conditions and it's effect has to be determined by the same method, taking into account the value resulting from the use of the detergent without cellulase. The value obtained for EG V is taken as AP = 100 %.

As can be seen from this definition, the known cellulase EG V from Humicola insolens has a ratio of TSL to AP of 1. As the above mentioned Bacillus cellulase available from Kao Corp. under the trade mark KAC® 500 or KAC® 700 has a low AP and a very low TSL, it can be seen that also the ratio for this cellulase is approximately 1. Cellulases which may be used according to the invention, especially in detergents, have a ratio of TSL to AP as much as possible below 1, preferably below 0.8 and more particularly in the range of 0.001 to 0.5. A ratio of TSL to AP of for example 0.5 means that only 50 % of tensile strength loss is seen at an enzyme concentration yielding the same antipilling effect as the standard cellulase.

The aqueous laundry solution preferably comprises cellulase according to the definition given above in concentrations of 0.01 mg/l to 0.2 mg/l, more particularly 0.015 mg/l to 0.1 mg/l. These concentrations refer to the weight of cellulolytic protein. In addition all ingredients normally found in laundry solutions can be present.

Another aspect of the present invention is the use of a single cellulase with a ratio of TSL to AP below 1 to provide an anti-greying effect to fabrics, especially coloured fabrics.

In another aspect of the invention a single cellulase with a ratio of TSL to AP below 1 is used to provide a softening effect to fabrics.

The present invention also relates to the use of a single cellulase with a ratio of TSL to AP below 1 to provide colour clarification or to inhibit colour deterioration of fabrics, especially coloured fabrics.

The present invention further relates to the use of a single cellulase with a ratio of TSL to AP below 1 to inhibit the wrinkling of fabrics and to ease the ironing of fabrics.

We found that the use of a single cellulase according to the definition of the invention, unlike previously known mixtures of cellulases which provide colour clarification, does not degrade cotton to an undesirable level causing tensile strength loss.

It is further found that in using a cellulase of the definition according to the invention, unlike previously known cellulases which provide colour clarification, the enzyme does not accumulate on the fabric after repeated laundry washing.

In another aspect, the invention is directed to detergent compositions, detergent additives and fabric softener compositions comprising a single cellulase according to the definition given above.

As noted before, the present invention generally relates to the use of novel cellulases. However, prior to disclosing this invention in more detail, the following terms will be defined:

„Cellulase" is a generic name for enzymes acting on cellulose and its derivatives, and hydrolysing them into glucose, cellobiose or cellooligosaccharides.

The term „single" cellulase used herein is intended to mean a cellulase which is produced by one gene.

„Obtainable from" an organism in connection with a cellulase means that such cellulase has an amino acid sequence which corresponds to the amino acid sequence of a cellulase which may be obtained from that organism.

„Derivative" is intended to indicate a protein which is derived from the native protein by addition of one or more amino acids to either or both the C- and N-terminal end of the native protein, substitution of one or more amino acids at one or a number of different sites in the native amino acid sequence, deletion of one or more amino acids at either or both ends of the native protein or at one or more sites in the amino acid sequence, or insertion of one or more amino acids at one or more sites in the native amino acid sequence. The preparation of a derivative is usually achieved by modifying a DNA sequence which encodes for the native protein, transformation of that DNA sequence into a suitable host and expression of the modified DNA sequence to form the derivative protein. The derivative of the invention includes peptides comprising altered amino acid sequences in comparison with a precursor enzyme amino acid sequence (e.g., a wild type or native state enzyme according to the present invention) and which peptides retain a characteristic enzyme nature of the precursor enzyme but which have altered properties in some specific aspect. For example, an altered cellulase may have an increased pH optimum or increased tempertature resistance but will retain its characteristic cellulase activity. Derivatives also includes chemical modifications of amino acid residues within the enzyme molecule.

„Host cell" means a cell which has the capacity to act as a host and expression vehicle for a recombinant DNA vector comprising DNA which encodes for the native protein or a derivative.

The term „cleaning" means the removal of dirt attached to laundry.

The term „pilling" in this respect is the formation of pills and fuzz on the surface of cotton containing fabrics due to broken or disordered fibres.

The term „antipilling" is used to describe the prevention of the formation of pills and fuzz on the surface of cotton containing fabrics as well as the removal of pills and fuzz from cotton containing fabrics. Antipilling normally results in colour clarification when coloured cotton containing fabrics are treated.

The term „colour clarification" in this respect is the reestablishment of the attractive fresh look of coloured fabrics containing or consisting of cellulose based fibres, which

have developed a greyish appearance by treatment, especially with laundry detergents, of the coloured fabric.

The term „redeposition" in this respect is deposition of dirt or colour components that were removed from these textiles or fabrics during a laundry washing or textile treatment.

The term „antiredeposition" in this respect is the action of cellulase to prevent or diminish the redeposition of dirt and colour components on the fabric.

By a „laundry solution" is meant an aqueous solution used for washing, rinsing or conditioning, e.g. softening, fabrics.

In a preferred aspect, the present invention relates to the use of a cellulase which is obtainable from microorganisms which are deposited according to the Budapest Treaty on the International Recognition of the Deposits of Microorganisms for the Purposes of Patent Procedures, at the Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands on December 23, 1993 under deposition numbers CBS 669.93 and CBS 670.93 (described in international patent application WO-A-95/18219). This strains have been classified as new species of the genus Bacillus, which do not belong to any of the presently known rRNA-groups of Bacillus. As used herein, the deposited species will be referred to as CBS 669.93 and CBS 670.93.

The microorganisms may be obtained for example from water and soil samples collected in alkaline environments such as alkaline soils and soda lakes.

The microorganisms have subsequently been screened using a carboxymethyl cellulose (CMC)-agar diffusion assay. Strains which showed a clearing zone in this test were isolated as potential cellulase producing strains. Genomic gene libraries of the alkali tolerant cellulase producing strains were constructed. Recombinant clones were screened by agar diffusion on CMC-agar. Recombinant clones that showed clearing zones around the colony were isolated. Single cellulases were produced by fermentation of the recombinant clones in 4*YEP-medium for 48 hours at 30°C. The obtained single cellulases, optionally purified as described in Example 1, were tested in the tests defined above to measure TSL and AP.

Surprisingly it was found that the cellulases obtainable from CBS 670.93 or CBS 669.93 show a good performance in both tests and have a ratio of TSL to AP below 1.

In a preferred embodiment of the invention, an approximately 50 kD cellulase (calculated on the basis of the amino acid sequence (SEQ ID No. 2) of the mature protein) derived from CBS 670.93 (referred to as „BCE 103" herein) is used. It has been revealed by analyzing the gene encoding the amino acid sequence of the approximately 50 kD cellulase that this cellulase is 89% identical in sequence and 92.5% similar in sequence to the cellulase CelA of *Bacillus sp.* N-4 (Fukumori et al., J. Bacter., vol. 168, pp. 479-485) by using the TFastA program (Sequence Analysis Software Package 6.0 of Genetic Computer Group. University of Wisconsin, Biotechnology Center, Madison, Wisconsin) as described by Pearson and Lipman in Proc. Nat. Acad. Sci., vol. 85, pp. 2444-2448 (1988). The amino acid sequence of BCE 113 is given in SEQ. ID. No. 3. The present invention further encompasses the use of cellulases with an amino acid sequence which have greater than 89 %, preferably greater than 95 % sequence identity and/or greater than 92.5 %, preferably greater than 97 % sequence similarity thereto, and detergents comprising such a cellulase.

In an equally preferred embodiment of the invention, an approximately 63 kD cellulase (calculated on the basis of amino acid sequence of the mature protein) derived from CBS 669.93 (referred to herein as „BCE 113") is used. It has been revealed by analyzing the gene encoding the amino acid sequence of the approximately 63 kD cellulase that this cellulase is 58 % identical in sequence and 72 % similar in sequence to the cellulase CelB of Bacillus lautus (Jorgensen et al, Gene, vol. 93 (1990), p. 55-60) by using the TFastA program (Sequence Analysis Software Package 6.0 of Genetic Computer Group, University of Wisconsin, Biotechnology Center, Madison, Wisconsin) as described by Pearson and Lipman in Proc. Nat. Acad. Sci., vol. 85 (1990), p. 2444-2448. The amino acid sequence of BCE 113 is given in SEQ ID No.3. The present invention further encompasses the use of cellulases with an amino acid sequence which have greater than 58 %, preferably greater than 80 % and more particularly greater than 90 % sequence identity and/or greater than 72 %, preferably greater than 80 % and more particularly greater than 90 % sequence similarity thereto, and detergents comprising such a cellulase.

A cellulase which may be used in detergents according to the present invention in addition to having a ratio of TSL to AP below 1 usually performs well in the Antiredeposition Test as described in Example 4. Whiteness maintenance of white fabric is measured by a reflectance measurement. The higher the reflectance value, the more effective the tested cellulase is in antiredeposition performance. They also perform well in the Softening Test as described in Example 4. Depilling is the removal of fibrils and/or microfibers that are disordered and/or broken which usually make a coloured cotton containing fabric look greyish. The more disordered and/or broken fibrils are removed the better the coloured cotton containing fabrics look. Depilling effectiveness can be judged by panels or can be quantified by an image analysis system, as specified above for the measurement of AP. Cellulases which fulfil the requirement of the ratio defined above usually exhibit the following properties: They show a delta REM of at least 4 units, preferably at least 5 units, in the Anti Redeposition Test as defined in the Examples, and they show a depilling result which is at least comparable to that of the cellulase obtainable from CBS 670.93.

The cellulases which can be used according to the present invention may be produced by a process which can be developed using genetic engineering. As a first step the gene encoding the cellulase of the present invention can be cloned using λ-phage (expression) vectors and E. coli host cells. Alternatively PCR cloning using consensus primers designed on conserved domains may be used. Expression of the gene encoding the cellulase of the present invention in E. coli is shown to give an active protein.

After a first cloning step in E. coli, a cellulase gene can be transferred to a more preferred industrial expression host such as Bacillus or Streptomyces species, a filamentous fungus such as Aspergillus, or a yeast. High level expression and secretion obtainable in these host organisms allows accumulation of the cellulase of the invention in the fermentation medium from which they can subsequently be recovered.

Cellulases according to the invention are preferably used in amounts of $8 \cdot 10^{-5}$ % by weight (0.8 ppm) to $8 \cdot 10^{-3}$ % by weight (80 ppm), more particularly $1 \cdot 10^{-4}$ % by weight (1 ppm) to $4 \cdot 10^{-3}$ % by weight (40 ppm), referring to the cellulolytic protein, in detergents. Detergent compositions comprising a cellulase defined according to the invention may additionally comprise surfactants which may be of the anionic, non-ionic,

cationic, amphoteric or zwitterionic type as well as mixtures of these surfactant classes. Detergent compositions of the invention may contain other detergent ingredients known in the art, as e.g. builders, bleaching agents, bleach activators, anti-corrosion agents, sequestering agents, soil release polymers, perfumes, other enzymes, enzyme stabilizers, etc.

Suitable builders are in particular those from the classes of polycarboxylic acids, more particularly polymeric acrylic acids, methacrylic acids, maleic acids, copolymers thereof and oxidized carbohydrates, as described in international patent application WO-A-93/16110, layer silicates, more particularly bentonites, alumosilicates, more particularly zeolites, crystalline or amorphous alkali metal silicates, more particularly sodium silicate, and alkali metal carbonates, more particularly sodium carbonate. The polycarboxylic acids mentioned are normally used in the form of their alkali metal salts, more particularly in the form of their sodium or potassium salts. The zeolites preferably incorporated are in particular those of the A, P or X type or mixtures thereof. Preferred alkali metal silicates are those with molar ratios of SiO_2 to alkali metal oxide of 1.5 to 3.0. Builders such as these are preferably present in detergents according to the invention in quantities of 20% by weight to 80% by weight.

Nonionic surfactants may be present in the detregents according to the invention, preferably in quantities of not more than 10% by weight and, more preferably, in quantities of 2% by weight to 6% by weight, based on the detergent as a whole. Suitable nonionic surfactants are alkyl polyglycosides containing 10 to 22 carbon atoms in the alkyl component and alkoxylates, particularly ethoxylates and/or propoxylates, of linear or branched C_{10-22} and preferably C_{12-18} alcohols. The degree of alkoxylation of the alcohols is between 1 and 20 and preferably between 3 and 10. They may be prepared in known manner by reaction of the corresponding alcohols with the corresponding alkylene oxides. The fatty alcohol derivatives are particularly suitable, although their branched-chain isomers, more particularly so-called oxoalcohols, may be used for the production of useable alkoxylates. Accordingly, the ethoxylates of primary alcohols containing linear dodecyl, tetradecyl, hexadecyl or octadecyl radicals and mixtures thereof are particularly useful. In addition, corresponding ethoxylation and/or propoxylation products of alkyl amines, vicinal diols and carboxylic acid amides, which correspond to the alcohols mentioned in regard to the alkyl component, and of alkyl phenols containing 5 to 12 carbon atoms in the alkyl component may also be used.

Suitable anionic surfactants are in particular those of the sulfate or sulfonate type, although other types, such as soaps, long-chain N-acyl sarcosinates, salts of fatty acid cyanamides or salts of ether carboxylic acids, which may be obtained from long-chain alkyl or alkyl phenyl polyglycol ethers and chloroacetic acid, may also be used. The anionic surfactants are preferably used in the form of the sodium salts. Surfactants are preferably present in quantities of 2% by weight to 30% by weight and more preferably in quantities of 5% by weight to 20% by weight.

Particularly suitable surfactants of the sulfate type are the sulfuric acid monoesters of long-chain primary alcohols of natural and synthetic origin containing 10 to 20 carbon atoms, i.e. the sulfuric acid monoesters of fatty alcohols such as, for example, coconut oil fatty alcohols, tallow fatty alcohols, oleyl alcohol, or the C_{10-20} oxoalcohols and those of secondary alcohols of the same chain length. The sulfuric acid monoesters of aliphatic primary alcohols, secondary alcohols and alkyl phenols ethoxylated with 1 to 6 mol ethylene oxide are particularly suitable. Sulfated fatty acid alkanolamides and sulfated fatty acid monoglycerides are also suitable.

The sulfonate-type surfactants are primarily the alkylbenzene sulfonates containing C_{9-15} alkyl groups, sulfosuccinic acid monoesters and diesters containing 6 to 22 carbon atoms in the alcohol components and the esters of a-sulfofatty acids, for example the a-sulfonated methyl or ethyl esters of hydrogenated coconut oil, palm kernel oil or tallow fatty acids. Other suitable surfactants of the sulfonate type are the alkane sulfonates obtainable from C_{12-18} alkanes by sulfochlorination or sulfoxidation and subsequent hydrolysis or neutralization or by addition of bisulfite onto olefins and also olefin sulfonates, i.e. mixtures of alkene and hydroxyalkane sulfonates and also disulfonates which are obtained, for example, from long-chain monoolefins with a terminal or internal double bond by sulfonation with gaseous sulfur trioxide and subsequent alkaline or acidic hydrolysis of the sulfonation products.

Bleaching agents are preferably selcted from the type containing peroxygen, as hydrogen peroxide, alkali perborate, alkali percarbonate, alkali persilicate and/or alkali persulfate. Particularly preferred are sodium perborate monohydrate and sodium percarbonate. Bleaching agents may be present in amounts of 5 % by weight to 25 % by weight, more particularly 7 % by weight to 20 % by weight.

Bleach activator compounds include in particular N- or O-acyl compounds, for example polyacylated alkylene diamines, more particularly tetraacetyl ethylene diamine, N-acylated triazines, more particularly 1,5-diacetyl-2,4-dioxohexahydro-1,3,5-triazine, acylated glycolurils, more particularly tetraacetyl glycoluril, N-acylated hydantoins, hydrazides, triazoles, urazoles, diketopiperazines, sulfuryl amides and cyanurates, also carboxylic anhydrides, more particularly phthalic anhydride, carboxylic acid esters, more particularly sodium isononanoyloxy benzene sulfonate, and acylated sugar derivatives, more particularly pentaacetyl glucose. The bleach activator may be coated in the usual way with shell-forming substances or may be granulated, optionally using granulation aids, and if desired may contain other additives, for example dye. A bleach activator which forms peroxocarboxylic acids with 2 to 12 carbon atoms, in particular peroxoacetic acid, under the washing conditions is preferably used. A particularly preferred bleach activator is tetraacetyl ethylene diamine (TAED) granulated with carboxymethyl cellulose with average particle sizes of 0.01 mm to 0.8 mm, which may be produced by the process described in European Patent EP-B-0 037 026. In addition to the above mentioned bleach activators or even subtituting them so-called bleach catalysts may be used, which are transition metal complexes, for example as described in

Enzymes which may be present in the detergents according to the invention, in addition to the cellulase according to the definition, are proteases, lipases, cutinases, amylases, pullulanases, other cellulases, hemicellulases, xylanases, oxidases and/or peroxidases. They may be presnt in amounts up to 5 % by weight, preferably 0.2 % by weight to 2 % by weight.

The detergent compositions of the invention may be formulated in any convenient form e.g. as a powder or liquid. For the production of detergents with high apparent density of e.g. 650 g/l to 950 g/l a method using an extrusion step, as described in European patent EP-B-0 486 592, is preferred.

Fabric softening compositions comprising the inventive cellulase may comprise further to this cellulase cationic surfactants, preferably of the so-called esterquat type, which are capable of fabric softening and which may increase the fabric softening properties of the compositions.

Examples

Example 1 Production of cellulases

- Screening for cellulase producing microorganisms

Two methods were applied for the isolation of cellulase-producing microorganisms:

1) the soil and water samples were suspended in 0.85% saline solution and directly used in the carboxymethyl cellulose (CMC)-agar diffusion assay for detection of cellulase producing colonies.

2) The soil and water samples were enriched for cellulase containing strains by incubation in a cellulose containing liquid minimal medium or GAM-medium for 1 to 3 days at 40°C. Cultures that showed bacterial growth were analyzed for cellulase activity using the CMC-agar diffusion assay for detection of cellulase producing colonies.

- Isolation of alkalitolerant, cellulase producing strains

Strains that showed clearing zones in the agar diffusion assay were fermented in 25 millilitre GAM-medium in 100 millilitre shake flasks in an Incubator Shaker (New Brunswick Scientific, Edison, NJ, USA), at 250 r.p.m. at 40°C for 72 hours. CMCase activity was determined in the culture broth at pH 9 and 40°C.

- Isolation of cellulase genes

Genomic gene libraries of the alkalitolerant cellulase producing strains were constructed in plasmid pTZ18R (Mead, D.A., et al. (1986) Protein Engineering 1, 67). Recombinant clones were screened by agar diffusion on CMC-agar as described by Wood, P.J., et al. (1988) Methods in Enzymology 160, 59-74. Strains that showed clearing zones around the colony were isolated. The CMCase activity of the recombinant strains was determined after fermentation for 48 hours at 30°C in 4*YEP-medium. The plasmid DNA of the recombinant strains was isolated and the inserts were characterized by restriction enzyme analysis and nucleotide sequence analysis.

- Media

The minimal medium (pH 9.7) used in the CMC-agar diffusion assay and the enrichment procedure, consisted of KNO_3 1%, Yeast extract (Difco) 0.1%, KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.02%, Na_2CO_3 1%, NaCl 4% and 0.25% CMC (Sigma C-4888). For solidificaton 1.5% agar was added.

The complex medium (GAM) used for enzyme production of the donor strains consisted of Peptone (Difco) 0.5%, Yeast extract (Difco) 0.5%, Glucose.H_2O 1%, KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.02%, Na_2CO_3 1%, NaCl 4%. The pH was adjusted to 9.5 with 4M HCl after which 1% CMC was added.

The complex medium (4*YEP) used for the enzyme production in E. coli recombinant strains consisted of Yeast extract (Difco) 4%, Peptone (Difco) 8%, lactose 0.2%, 100 µg/ml ampicilline.

- CMC-agar diffusion assay for colonies

Cell suspensions in 0.85% saline solution were plated on CMC-containing minimal medium. After incubation for 1 to 3 days at 40°C, the plates were replica plated and the parent plate was flooded with 0.1% Congo Red for 15 minutes. The plates were destained with 1M NaCl for 30 minutes. The strains that showed a clearing zone aroung the colony were isolated as potential cellulases producing microorganisms.

- CMC-agar diffusion assay for liquid fractions

Aliquots of 40 µl of enzyme solution or fermentation broth were pipetted in wells punched out from a layer of 5 mm of minimal medium in a petri dish. After incubation for 16 hours at 40°C cellulase activity was detected by Congo Red / NaCl treatment. The diameter of the clearing zone is a measure for the CMCase activity.

- Resulting cellulase

These experiments resulted in the isolation of a cellulase producing microorganism which was deposited thereafter as CBS 670.93. The microorganism was classified as a

new species of the genus Bacillus. Cloning experiments with the CBS 670.93 strain as a donor strain resulted in the isolation of an E. coli clone which was able to produce a cellulase called BCE 103. The nucleotide sequence of the gene coding for said cellulase was analysed. From the cellulase BCE 103 the N-terminal amino acid sequence was determined using standard methods for obtaining and sequencing peptides (Finlay & Geisow (Eds.), Protein Sequencing - a practical approach, 1989, IRL Press). The amino acid sequence of the cellulase was deduced from the nucleotide sequence, using the N-terminal amino acid sequence for the starting point of the mature protein.

The nucleotide sequence for BCE 103 is shown in SEQ ID No. 1 and the amino acid sequence is shown in SEQ ID No. 2.

- Purification of the cellulase

After the fermentation the cells were separated from the culture liquid by centrifugation (8000 rpm). The cellulase in the supernatant was precipitated with ammonium sulphate (65% saturation). The precipitate was dissolved in 25 mM phosphate buffer pH 7 + 5 mM EDTA until a conductivity of 7 mS/cm. This solution was applied to a Q-Sepharose FF (diameter 5 cm, length 10 cm) Anion Exchange column, after which the column was washed with 25 mM phosphate buffer pH 7 + 5 mM EDTA until an absorbency of 0.2 AU. A gradient of 0 to 0.5 M NaCl in 25 mM phosphate pH 7 was applied to the column in 80 minutes followed by a gradient from 0.5 to 1 M NaCl in 10 minutes. Depending on which cellulase was applied to the column, elution took place in the first or the second gradient. After elution the column was cleaned (upflow) with 1 M NaOH and equilibrated again with 25 mM phosphate pH 7 + 5 mM EDTA. Depending on the elution the obtained cellulase had a purity of up to about 80%.

- Characterization

CMC'ase assay

Assays for cellulase activity were performed using modified methods of the PAHBAH method (Lever M. Anal. Biochem. 1972, 47, 273-279 and Lever M. Anal. Biochem. 1977, 81, 21-27).

Procedure

A test tube is filled with 250 µl 2.5% CMC in 50 mM glycine buffer pH 9 (CMC-low viscosity is purchased from Sigma) and 250 µl aliquots cellulase, diluted in the appropriate buffer. The test tube is incubated for 30 minutes at 40°C in a waterbath, whereafter 1.5 ml of a daily fresh prepared PAHBAH solution (1% PAHBAH in 100 ml 0.5 M NaOH with 100 µl bismuth solution (containing 48.5 g bismuth nitrate, 28.2 g potassium sodium tartrate and 12.0 g NaOH in 100 ml) is added. The mixture is heated at 70°C for 10 minutes, after which it is cooled on ice for 2 minutes. The absorption is measured at 410 nm. To eliminate the background absorbance of the enzyme samples a control experiment is executed as follows: a tube with substrate is incubated under the same conditions as the test tube. After the incubation 1.5 ml PAHBAH and the enzyme preparation is added (in this order). One unit (U) is defined as the amount of enzyme producing 1 µmol of glucose from CMC equivalent determined as reducing sugars per minute per gram product.

The buffer used for the determination of the pH/temperature profiles is a phosphate/citrate system. The pH/temperature profiles were determined using a fixed enzyme concentration which fits in the linear range of the dose response profile measured at pH 7 and 40°C. This enzyme concentration was used for the measurement of the activities under all other determined conditions.

The results for the cellulase BCE 103 are shown in Figure 1. This cellulase shows good activities at alkaline pH, which makes it suitable for application in detergents with an alkaline pH.

Example 2

Similar procedures starting with the alkalophilic bacillus strain CBS 669.93 resulted in cellulase BCE 113. The results for this cellulase BCE 113 are shown in Figure 2. This cellulase also shows good activities at alkaline pH, which makes it suitable for application in detergents with an alkaline pH.

Example 3: Measurement of tensile strength and antipilling

As described for the evaluation of TSL, washing experiments were performed using as detergent matrix a Colour Detergent without bleach, without perfume and enzymes (105 g detergent per wash cycle, pH 10.5), as washing machine a type Miele® W 717, temperature 40 °C, program „Normalprogramm", with water of a hardness of 16 °dH (German hardness), wash load 3.5 kg, 25 washes.

Experiments using a composition according to the invention (**D1**) as well as comparisons (**C1** to **C3**) were run in parallel in identical machines:

C1: detergent matrix without cellulase

C2: detergent matrix + 0.288 mg endoglucanase V from *Humicola insolens*

C3: detergent matrix + cellulase mixture from *Humicola insolens* sold as granules Celluzyme® 0.7T

D1: detergent matrix + 0.288 mg cellulase BCE 103

D2: detergent matrix + 0.288 mg cellulase BCE 113

Table 1: Results of TSL-measurements [%]

Composition	TSL
C1	0
C2	100
C3	38
D1	12

Using washing machines of type Miele® W 914, under otherwise identical conditions, gave the following results:

Table 2: Results of TSL-measurements [%]

Composition	TSL

C1	0
C2	100
D2	0.6

Example 3: Measurement of antipilling and Calculation of the ratio TSL to AP

The evaluation of antipilling properties was done with increased concentrations of cellulases for better quantitatve evaluation of the effect. A Colour Detergent (5g/l, 10 wash cycles at 40 °C) with the addition of cellulase as given in Table 3 was used on „pilled" sweat shirt cotton material (washed 25 times at 60 °C with a detergent without cellulase). Evaluation of the pilling was done with the optical measurement system as described before; a degree of pilling of 0 % was assigned to the „pilled" material.

Table 3: Results of AP-measurements [%]

Enzyme concentration	degree of pilling		AP [%]of BCE 103
	EG V	BCE 103	
25 µg/ml	-12.8%	-8.4 %	65 %
37,5µg/ml	-16.0%	-9.6 %	59 %
50 µg/ml	-22,8 %	-15.6 %	68 %

An average AP of 64% can be calculated for BCE 103 cellulase. BCE 113 cellulase showed under the same conditions an average AP of 100 %.

Using the values for TSL in Tables 1 and 2, the ratios of TSL to AP for the various cellulases are as in the following Table 4:

Table 4: Ratio TSL to AP

Enzyme	Ratio
EG V	1
BCE 103	≈ 0.2
BCE 113	≈ 0.02

Example 4: Further test procedures

- Anti redeposition test

20 ml 0.5% pigmented soil (fresh prepared, daily and consisting of 86% kaolin, 8% soot (Flammruß 101, obtained from Degussa AG), 4% iron oxide black and 2% iron oxide yellow (from Henkel Genthin GmbH)), in a detergent (Persil color® without enzymes, 5 g/l, pH 8.5) was, under agitating (90 rpm) incubated with white cotton fabric (prewashed, 5 cm diameter, obtained from Windelbleiche, Krefeld). Cellulase was added until a final concentration of 1 mU/ml. The mixture was incubated for 30 minutes at 40°C, 90 rpm. As a control the same incubation was carried out without the addition of cellulase. After the incubation the fabric was rinsed thoroughly with running cold water. After drying the whiteness of the fabric was measured by remission (4 measurements per fabric) using a Micro colour Dr. Lange® Colourimeter. The control value was substracted from the sample value. The results, expressed as delta Rem, are shown in Table 5.

-Fibre Damage Test

One pad of cotton wool (100% cotton, Warenhandels GmbH, Buchholz, Marke Olivia, Selling agency: Aldi) was incubated in 40 ml wash liquor (Persil color® without enzyme, 5 g/l pH 8.5), cellulase at a final concentration of 1 mU/ml was added in a sealed flask and incubated for 20 hours at 40°C under agitation (90 rpm). After the incubation, fibre damage was monitored by the measurement of the quantity of the reducing sugars in solution, using the PAHBAH method described in Example 1. As a control the same incubation was carried out without the addition of cellulase. The results are shown in Table 5.

-Adsorption Test

White cotton fabric (Windelbleiche, Bielefeld) prewashed with german Persil® without enzymes at 60°C, was cut round to 9 cm diameter (approx. 0.920 gram). One cotton swatch was incubated in 50 ml 50 mM glycine-NaOH buffer pH 9 including 0.1% SDS and 1 ml cellulase sample (600 mU/ml) for 60 minutes at 30°C. 2 ml samples were taken at T=0 and at T=60 minutes and were diluted directly (1:2) with 50 mM MES-buffer pH 6.5 and stored at 4°C until measurement. As control the same incubation was carried out without the addition of cotton textile. The activity measurement was determined with a PAHBAH method as described in Example 3, but at pH 6.5 in 50 mM MES buffer. The adsorption was expressed as relative adsorption where the activity applied at the start of the experiment was set as 100%, T=0. 100% activity value - remaining activity (%) = adsorption (%). The results are shown in Table 5.

Table 5: Results of the Antiredeposition Test, Fibre Damage Test and Adsorption Test

Enzyme	Antiredeposition [delta REM]	Fibre Damage [mU]	Adsorption [%]
BCE 103	5.0	0.025	7
KAC®[a)]	7.5	0.006	0
EG V	1.2	0.155	36

a): Cellulase of Kao Corporation

Cellulase BCE 113 performed in theses tests at least as well as cellulase BCE 103.

-Softening test

The softness of fabrics treated as in Example 3, but after 15 wash cycles, was rated by an expert panel (5 persons) who awarded grades between 0 (fabric washed 25 times with a detergent without cellulase) and 6 (fabric prior to any wash) by the feel of the fabrics. Compositions as defined in Example 2 were used in the washings. The average rates are

given in Table 6. It can be seen that the compositions according to the invention showed the best performance.

Table 6: Results of the Softening Test

Composition	Rate
C1	0
C2	2.1
C3	1.5
D1	2.3
D2	2.2

Legend to the figures

Figure 1 shows the relative activities of the cellulase BCE 103. In Example 1 this figure is referred to as the pH/temperature profiles. All activities for both 40 and 60°C are related to the highest activity which is fixed on 100%.

Figure 2 shows the relative activities of the cellulase BCE 113.

Figure 3 shows the DNA sequence (SEQ ID No. 1) and deduced amino acid sequence (SEQ ID No. 2) of the 50 kD cellulase derived from CBS 670.93 with the leader peptide sequence shaded, which upon secretion is cleaved to yield the mature enzyme.

Figure 4 shows the DNA sequence (SEQ ID No. 4) and deduced amino acid sequence (SEQ ID No. 3) of the 63 kD cellulase derived from CBS 669.93 with the leader peptide sequence underlined, which upon secretion is cleaved to yield the mature enzyme.

Claims

1. Use of a single cellulase with a ratio of tensile strength loss (TSL) to antipilling properties (AP) below 1 in aqueous laundry solutions.

2. Use according to claim 1, characterized in that the aqueous laundry solution comprises the cellulase in concentrations of 0.01 mg/l to 0.2 mg/l, more particularly 0.015 mg/l to 0.1 mg/l.

3. Use of a single cellulase with a ratio of TSL to AP below 1 to provide an anti-greying effect to fabrics, especially coloured fabrics.

4. Use of a single cellulase with a ratio of TSL to AP below 1 to provide a softening effect to fabrics.

5. Use of a single cellulase with a ratio of TSL to AP below 1 to provide colour clarification to fabrics or to inhibit colour deterioration of fabrics, especially coloured fabrics.

6. Use of a single cellulase with a ratio of TSL to AP below 1 to inhibit the wrinkling of fabrics and to ease the ironing of fabrics.

7. Use according to any of claims 1 to 6, characterized in that the ratio of TSL to AP is below 0.8 and more particularly in the range of 0.001 to 0.5.

8. Use according to any of claims 1 to 7, characterized in that the cellulase is obtainable from Bacillus sp. CBS 669.93 or CBS 670.93.

9. Use according to any of claims 1 to 8, characterized in that the cellulase has the amino acid sequence as listed in SEQ ID No. 2 or a derivative thereof.

10. Use according to any of claims 1 to 8, characterized in that the cellulase has the amino acid sequence as listed in SEQ ID No. 3 or a derivative thereof.

11. Use according to claim 9 or 10, characterized in that the cellulase has an amino acid sequence with greater than 58 %, preferably greater than 80 % and more particularly greater than 90 % sequence identity and/or greater than 72 %, preferably greater than 80 % and more particularly greater than 90 % sequence similarity to the amino acid sequence as listed in SEQ ID No. 2 and/or SEQ ID No. 3.

12. A detergent composition which comprises a single cellulase with a ratio of TSL to AP below 1.

13. A detergent composition according to claim 12, characterized in that it comprises 0.8 ppm to 80 ppm, more particularly 1 ppm to 40 ppm of the cellulase.

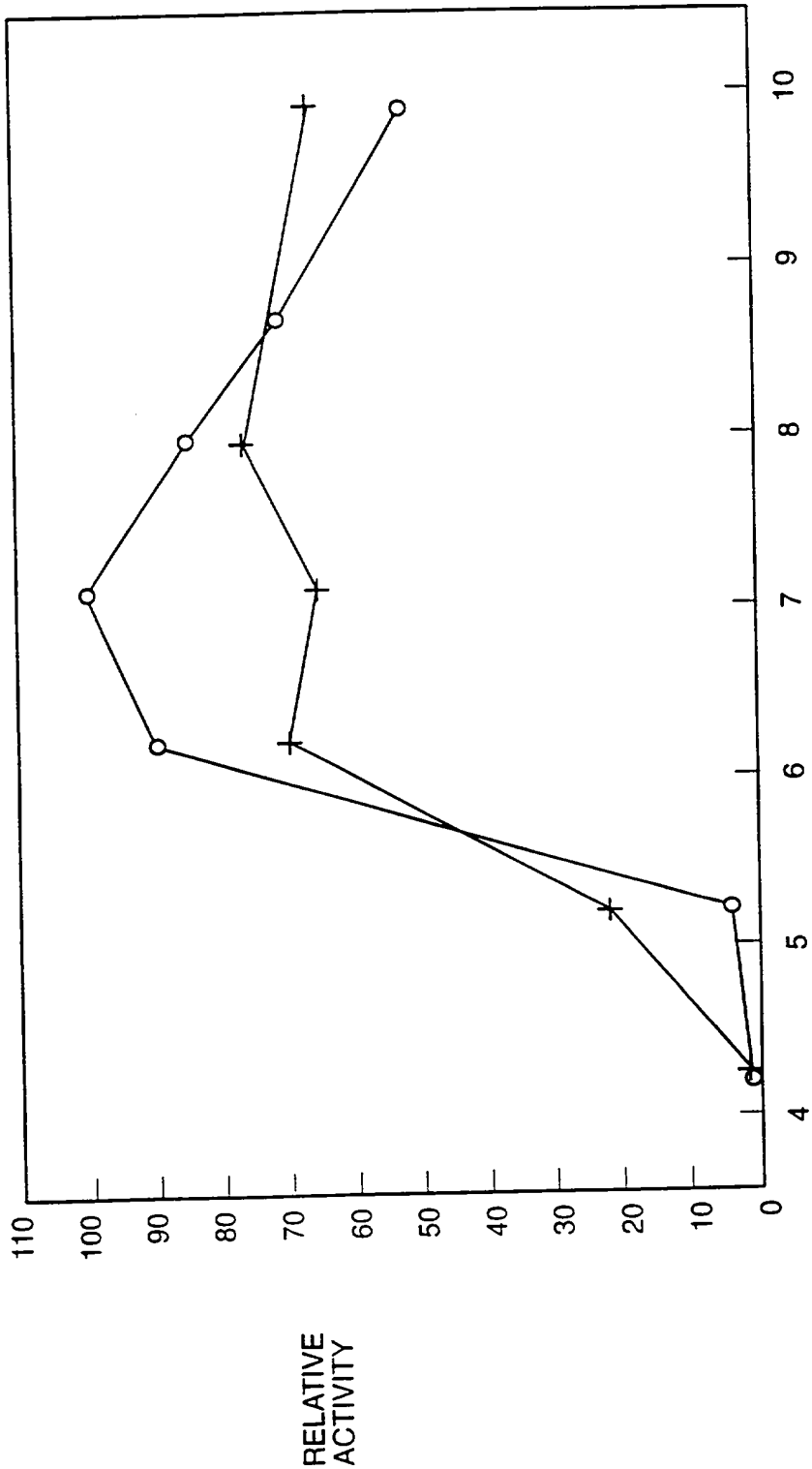

FIG._1

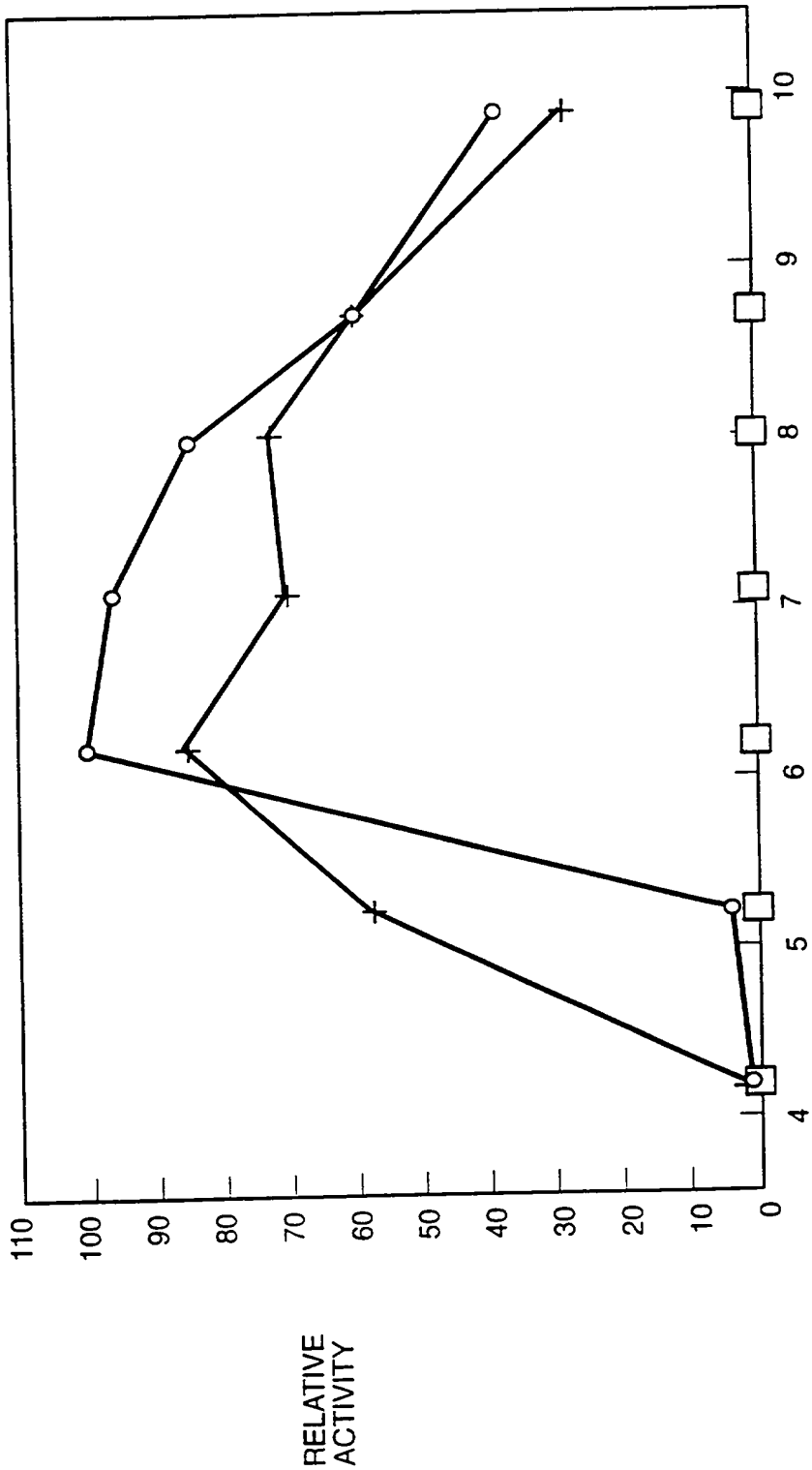

FIG._2

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-121 GAATTCCGTTACATATTTTGCAAAAAAGAGGGTGGTGGCGCTACATATACACCTTAAAAAG

 -60 TGCAGACTAAAACGATTTCGTTTCAGTATGAAAAGCTAAACCATTACCAAGGAGGAAATT

   1 ATGAAAAAGATAACTACTATTTTTGCCGTATTGCTCATGACATTGGCGTTGTTCAGTATA
     MetLysLysIleThrThrIlePheAlaValLeuLeuMetThrLeuAlaLeuPheSerIle

  61 GGAAACACGACAGCGGCTGATGATTATTCAGTTGTAGAGGAACATGGGCAACTAAGTATT
     GlyAsnThrThrAlaAlaAspAspTyrSerValValGluGluHisGlyGlnLeuSerIle

 121 AGTAACGGTGAATTAGTCAATGAACGAGGCGAACAAGTTCAGTTAAAAGGGATGAGTTCC
     SerAsnGlyGluLeuValAsnGluArgGlyGluGlnValGlnLeuLysGlyMetSerSer

 181 CATGGTTTGCAATGGTACGGTCAATTTGTAAACTATGAAAGCATGAAATGGCTAAGAGAT
     HisGlyLeuGlnTrpTyrGlyGlnPheValAsnTyrGluSerMetLysTrpLeuArgAsp

 241 GATTGGGGAATAACTGTATTCCGAGCAGCAATGTATACCTCTTCAGGAGGATATATTGAC
     AspTrpGlyIleThrValPheArgAlaAlaMetTyrThrSerSerGlyGlyTyrIleAsp

 301 GATCCATCAGTAAAGGAAAAAGTAAAAGAGACTGTTGAGGCTGCGATAGACCTTGGCATA
     AspProSerValLysGluLysValLysGluThrValGluAlaAlaIleAspLeuGlyIle

 361 TATGTGATCATTGATTGGCATATCCTTTCAGACAATGACCCGAATATATATAAAGAAGAA
     TyrValIleIleAspTrpHisIleLeuSerAspAsnAspProAsnIleTyrLysGluGlu

 421 GCGAAGGATTTCTTTGATGAAATGTCAGAGTTGTATGGAGACTATCCGAATGTGATATAC
     AlaLysAspPhePheAspGluMetSerGluLeuTyrGlyAspTyrProAsnValIleTyr

 481 GAAATTGCAAATGAACCGAATGGTAGTGATGTTACGTGGGACAATCAAATAAAACCGTAT
     GluIleAlaAsnGluProAsnGlySerAspValThrTrpAspAsnGlnIleLysProTyr

 541 GCAGAAGAAGTGATTCCGGTTATTCGTGACAATGACCCTAATAACATTGTTATTGTAGGT
     AlaGluGluValIleProValIleArgAspAsnAspProAsnAsnIleValIleValGly

 601 ACAGGTACATGGAGTCAGGATGTCCATCATGCAGCCGATAATCAGCTTGCAGATCCTAAC
     ThrGlyThrTrpSerGlnAspValHisHisAlaAlaAspAsnGlnLeuAlaAspProAsn

 661 GTCATGTATGCATTTCATTTTTATGCAGGAACACATGGACAAAATTTACGAGACCAAGTA
     ValMetTyrAlaPheHisPheTyrAlaGlyThrHisGlyGlnAsnLeuArgAspGlnVal

 721 GATTATGCATTAGATCAAGGAGCAGCGATATTTGTTAGTGAATGGGGGGACAAGTGCAGCT
     AspTyrAlaLeuAspGlnGlyAlaAlaIlePheValSerGluTrpGlyThrSerAlaAla

 781 ACAGGTGATGGTGGTGTGTTTTTAGATGAAGCACAAGTGTGGATTGACTTTATGGATGAA
     ThrGlyAspGlyGlyValPheLeuAspGluAlaGlnValTrpIleAspPheMetAspGlu

 841 AGAAATTTAAGCTGGGCCAACTGGTCTCTAACGCATAAGGATGAGTCATCTGCAGCGTTA
     ArgAsnLeuSerTrpAlaAsnTrpSerLeuThrHisLysAspGluSerSerAlaAlaLeu

 901 ATGCCAGGTGCAAATCCAACTGGTGGTTGGACAGAGGCTGAACTATCTCCATCTGGTACA
     MetProGlyAlaAsnProThrGlyGlyTrpThrGluAlaGluLeuSerProSerGlyThr
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FIG._3A

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961  TTTGTGAGGGAAAAAATAAGAGAATCAGCATCTATTCCGCCAAGCGATCCAACACCGCCA
     PheValArgGluLysIleArgGluSerAlaSerIleProProSerAspProThrProPro

1021 TCTGATCCAGGAGAACCGGATCCAGGAGAACCGGATCCAACGCCCCCAAGTGATCCAGGA
     SerAspProGlyGluProAspProGlyGluProAspProThrProProSerAspProGly

1081 GAGTATCCAGCATGGGATTCAAATCAAATTTACACAAATGAAATTGTGTATCATAACGGT
     GluTyrProAlaTrpAspSerAsnGlnIleTyrThrAsnGluIleValTyrHisAsnGly

1141 CAGTTATGGCAAGCGAAATGGTGGACACAAAATCAAGAGCCAGGTGACCCATACGGTCCG
     GlnLeuTrpGlnAlaLysTrpTrpThrGlnAsnGlnGluProGlyAspProTyrGlyPro

1201 TGGGAACCACTCAAATCTGACCCAGATTCAGGAGAACCGGATCCAACGCCCCCAAGTGAT
     TrpGluProLeuLysSerAspProAspSerGlyGluProAspProThrProProSerAsp

1261 CCAGGAGAGTATCCAGCATGGGATTCAAATCAAATTTACACAAATGAAATTGTGTACCAT
     ProGlyGluTyrProAlaTrpAspSerAsnGlnIleTyrThrAsnGluIleValTyrHis

1321 AACGGCCAGCTATGGCAAGCAAAATGGTGGACACAAAATCAAGAGCCAGGTGACCCATAT
     AsnGlyGlnLeuTrpGlnAlaLysTrpTrpThrGlnAsnGlnGluProGlyAsnProTyr

1381 GGTCCGTGGGAACCACTCAATTAAACTATATAATTGATAAAAATTTACTAATGAGATAGT
     GlyProTrpGluProLeuAsnEnd

1441 GAGAATCCCAAGAGTCTAAATTTGAAGATTGGCATTCTCATTTTACAATTAATTTAATCC

1501 ATTGAAAATATTTAAAAACGAATTTTATAATATCCAAGGTACCATACTTAATTGGCGGTA

1561 CTTTTTTCTGTCCTTATAGCTGCCCATCCCCCCGAAAAAGCGGTCGAAAACTGGTGCATT

1621 TTTCAGCATTATCTTGTAAATATCAAAACATAAGAAAAAGCCTTGAAACATTGATATGAC

1681 AACGTTTCTAAGGCTTTTCTGCATTTCTTATTCAGTGTATGCCAATTAACGAGAGTACCA

1741 CTCAACGATAAGTTGTTCGTTAATTTCAGCTGGAAGCTCAGAACGCTCAGGTAAACGAGT

1801 GAACGTACCTTCAAGCTT
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FIG._3B

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-630                                                GAATTCTTTGGATCATGATGGAAGGCGAAA

-600 TCATGAGCATTGCCCTTGCGACGATTACGGCTTCTGTCGGCGTCTACTTGCTTGCGTCAG

-540 CGGTTCAAGGTTGGTTTGCAGGTAAAGCTGCATTAACTGTTGTTCGTTTACTTCTCATTG

-480 TCGCTGCTGTTTGTCTTATTCATTCAAATTGGGTGTATGACTTTGTCGCCCTCGGNATCG

-420 CGGGTATCGCCATTATNCTTCAAAGAACAGTTATTAACAGACGCCATGGGTTCCAAGGCA

-360 AGTACAGTTTAAAACGAGAGATTTAAGAGGCCGCTCCCAATGAGGGAGTGGTCTTTTTTA

-300 CATTCNAAAAAGAGGAAAATAGGAGAAATGTAGATCCGACGTAGATAAGTATTAGGTTTT

-240 AAGTGTAAGTACAGCTAAGAAAGCTGCTTTTGCTGATTCTATGAAAAAGTGCTTGTTAAA

-180 CATTTTGACATGATTTTCTGTGAAATAAATGATCTATTTTCTGTGAAACAATTGTGATAG

-120 ATTGGTGTAGAGTTTTGATAATTCTAAATTTTCGTTCAAAAGGAGGTTGAGGTTCATTTA

 -60 CGATTTTGTCAACAGTCAATTGTTGTTTCCGGGTAACTCATTTGGAGGTGGTGGAGTCTG

   1 ATGAAGTGGATGAAATCCATGGTATGGTTGGCCGTTGTTTTGGTCGTTTCGTTCGTAGCT
     MetLysTrpMetLysSerMetValTrpLeuAlaValValLeuValValSerPheValAla

  61 CCTGCCGTTAGTTCAGCTAATGAGGATGTAAAAACTCTCGATATTCAGTCCTATGTAAGA
     ProAlaValSerSerAlaAsnGluAspValLysThrLeuAspIleGlnSerTyrValArg

 121 GACATGCAGCCGGGTTGGAATCTTGGGAATACGTTTGATGCCGTCGGACAAGATGAAACA
     AspMetGlnProGlyTrpAsnLeuGlyAsnThrPheAspAlaValGlyGlnAspGluThr

 181 GCATGGGGAAATCCACGTGTGACACGAGAATTAATTGAACGGATTGCGGATGAAGGGTAT
     AlaTrpGlyAsnProArgValThrArgGluLeuIleGluArgIleAlaAspGluGlyTyr

 241 AAAAGCATTCGGATTCCGGTGACGTGGGAAAATCGTATCGGAGGGGCACCTGATTATCCT
     LysSerIleArgIleProValThrTrpGluAsnArgIleGlyGlyAlaProAspTyrPro

 301 ATTGATCCCCAGTTTTTAAATCGAGTGGACGAAGTTGTTCAATGGGCGCTGGAAGAAGAT
     IleAspProGlnPheLeuAsnArgValAspGluValValGlnTrpAlaLeuGluGluAsp

 361 TTGTATGTCATGATTAATTTACACCATGATTCATGGTTATGGATTTATGAAATGGAGCAC
     LeuTyrValMetIleAsnLeuHisHisAspSerTrpLeuTrpIleTyrGluMetGluHis

 421 AACTACAACGGTGTGATGGCCAAGTATCGCTCGCTCTGGGAGCAACTATCGAACCACTTC
     AsnTyrAsnGlyValMetAlaLysTyrArgSerLeuTrpGluGlnLeuSerAsnHisPhe

 481 AAAGACTATCCAACAAAGCTTATGTTTGAAAGTGTCAATGAGCCAAAGTTTAGTCAAAAC
     LysAspTyrProThrLysLeuMetPheGluSerValAsnGluProLysPheSerGlnAsn

 541 TGGGGGTGAGATCCGTGAGAATCACCATGCGTTACTAGACGACTTAAACACAGTGTTTTTC
     TrpGlyGluIleArgGluAsnHisHisAlaLeuLeuAspAspLeuAsnThrValPhePhe

 601 GAGATTGTGAGACAGTCTGGTGGCCAAAATGATATCCGGCCGTTAGTGTTACCGACTATG
     GluIleValArgGlnSerGlyGlyGlnAsnAspIleArgProLeuValLeuProThrMet

 661 GAAACAGCCACATCACAACCGTTGCTGAACAACCTTTATCAAACAATTGACAAATTGGAT
     GluThrAlaThrSerGlnProLeuLeuAsnAsnLeuTyrGlnThrIleAspLysLeuAsp
```

FIG._4A

```
721 GATCCGAATCTAATTGCGACAGTACACTATTACGGGTTTTGGCCTTTTAGCGTGAATATC
    AspProAsnLeuIleAlaThrValHisTyrTyrGlyPheTrpProPheSerValAsnIle

781 GCCGGCTACACTCGCTTTGAAGAGGATTCGAAACGGGAGATCATCGAAACGTTTGATCGA
    AlaGlyTyrThrArgPheGluGluAspSerLysArgGluIleIleGluThrPheAspArg

841 GTACACCATACATTTGTTGCAAGAGGGATTCCAGTCGTTTTAGGTGAGTTCGGCTTGCTT
    ValHisHisThrPheValAlaArgGlyIleProValValLeuGlyGluPheGlyLeuLeu

901 GGATTTGATAAACATACTGGAGTGATTCAACAAGGTGAAAAGCTAAAATTCTTTGAGTAT
    GlyPheAspLysHisThrGlyValIleGlnGlnGlyGluLysLeuLysPhePheGluTyr

961 CTCATCCATCATTTGAACGAGCGGGATATTACTCATATGCTTTGGGATAATGGGCAGCAT
    LeuIleHisHisLeuAsnGluArgAspIleThrHisMetLeuTrpAspAsnGlyGlnHis

1021 TTCAATCGTCATACGTACGAATGGTATGACGAGGAATTGTTTGACATGTTGCGGGCAAGC
     PheAsnArgHisThrTyrGluTrpTyrAspGluGluLeuPheAspMetLeuArgAlaSer

1081 TGGGGAGGAAGATCATCCGTTGCAGAGTCGAACTTTATCTATTTAAAACAGGGAGACCGA
     TrpGlyGlyArgSerSerValAlaGluSerAsnPheIleTyrLeuLysGlnGlyAspArg

1141 ATCGCAGATGCAACAGTTACATTACAATTGCACGGAAATGAATTAACAGGGCTTCAGGCG
     IleAlaAspAlaThrValThrLeuGlnLeuHisGlyAsnGluLeuThrGlyLeuGlnAla

1201 AATGGACAACGACTAACGCCGGGGCAGGACTATGAGTTAAATGGAGAAAGACTTACAGTG
     AsnGlyGlnArgLeuThrProGlyGlnAspTyrGluLeuAsnGlyGluArgLeuThrVal

1261 AAGGCCCATGTCCTATCGGCAATCGCAGGTTCAGGTACGTTAGGTACGAATGGAATGGTA
     LysAlaHisValLeuSerAlaIleAlaGlySerGlyThrLeuGlyThrAsnGlyMetVal

1321 ACGGCTGAGTTTAATCGTGGGGCAGATTGGCATTTTCGGGTGAATACGTATCGTACGCCT
     ThrAlaGluPheAsnArgGlyAlaAspTrpHisPheArgValAsnThrTyrArgThrPro

1381 GTATTGCAAAGCACGCAAGGTCACGTGAGCAACTTCAGCATTCCTGCTTCCTTTAATGGG
     ValLeuGlnSerThrGlnGlyHisValSerAsnPheSerIleProAlaSerPheAsnGly

1441 AATAGCTTAGCAACAATGGAGGCTGTCTATGTGGATGGCGGAAATGCTGGCCCGCAAGAC
     AsnSerLeuAlaThrMetGluAlaValTyrValAspGlyGlyAsnAlaGlyProGlnAsp

1501 TGGACCTCCTTTAAGGAGTTTGGCTATGCCTTCTCTCCTTCTTATGATACACATGAGATT
     TrpThrSerPheLysGluPheGlyTyrAlaPheSerProSerTyrAspThrHisGluIle

1561 AAACTGACCGAGGCGTTTTTTCGTGAGGTGCGGGATGGTGAAGTTCGGTTAACCTTCCAT
     LysLeuThrGluAlaPhePheArgGluValArgAspGlyGluValArgLeuThrPheHis

1621 TTTTGGAGTGGTGAAATAGTCAACTATACGATTATTAAAAACGGGAACCAGGTGACTGGG
     PheTrpSerGlyGluIleValAsnTyrThrIleIleLysAsnGlyAsnGlnValThrGly

1681 ATAGCAGCTCAGACAACCAATTCAAAAAACAAAAATAAAAAATGAAATTGAAAGCGCTTT
     IleAlaAlaGlnThrThrAsnSerLysAsnLysAsnLysLysEnd

1741 CTATGGTGTTGCCCGAATATCTGAGGTTCTTTAGTAGAATCCGATATTCGGGTTTTTTCA

1801 TACATTATAGGGGCGCTTTTTTATGTTGCGCAGGTTAAATGGTCTTACGTATGGGAACCC

1861 TACTACTAGATTATTGTGCACTCTTTTTGAGTACCATTATCACCGCCCTATCATATGTAT
```

FIG._4B

```
1921 ATGAGTTGAACCATCTAGTAACCTCTCTTAAAATTGGTAAAGGAAATGTAACGTTGTGAT
2041 AGTAAGGAAATGGTATGATGGAGAGAGACGTGTGATCGAGAAATGGAGGAACGCAGAATG
2101 AATGAAACGATGCAACGCATCGCGAGAGTCATAGAGAATGTGGAACGAGTGGCCGCCGGG
2161 AAACGTCAGGAAATCGAGCTGAGCCTTGTCGCATTATTTGCTAGCGG
```

FIG._4C

"Express Mail" mailing label number TB811364664US. Date of Deposit February 27, 1998.

#4

PTO/SB/01 (6-95)
Approved for use through: 10/31/98 OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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U.S. Department of Commerce
Patent and Trademark Office

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing

Attorney Docket Number	H 1920 PCT/US
First Named Inventor	Lenting, Hermanus Bernardus Maria
COMPLETE IF KNOWN	
Application Number	08/945,574
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:
My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DETERGENTS COMPRISING CELLULASES

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 04/26/1996 as United States Application Number or PCT International Application Number PCT/EP96/01755 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
95201115.3	Europe	04/28/1995	☐	☐	☒
614,115	US	03/12/1996	☐	☐	☒

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
		☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

Burden Hour Statement: This form is estimated to take .4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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DECLARATION

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112.1 acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
	PCT/EP96/01755	04/26/1996	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Firm Name _____ Customer Number or label _____

OR

☒ List Attorney(s) and/or agent(s) name and registration number below:

Name	Registration Number	Name	Registration Number
Ernest G. Szoke	22,135	John E. Drach	32,891
Wayne C. Jaeschke	21,062	Martin G. Meder	34,674
Real J. Grandmaison	25,981	Glenn E. J. Murphy	33,539
Norvell E. Wisdom, Jr.	30,510		

☐ Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto.

Please direct all correspondence to: ☐ Customer Number or label _____ OR ☒ Fill in correspondence address below

Name	Glenn E. J. Murphy				
Address	Henkel Corporation				
Address	140 Germantown Pike, Suite 150				
City	Plymouth Meeting	State	PA	ZIP	19462
Country	USA	Telephone	610-832-2228	Fax	610-941-6067

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned

Given Name	**Hermanus Bernardus Maria**	Middle Initial		Family Name	**Lenting**	Suffix e.g. Jr.	
Inventor's Signature	H.B.M. Lenting (Herman Lenting)			Date	February 6, 1998		
Residence: City	**VT Pijnacker**	State		Country	**The Netherlands**	Citizenship	**The Netherlands**
Post Office Address	**Hunze 5**						
Post Office Address							
City	**2641 VT Pijnacker**	State		Zip		Country	**The Netherlands**
Applicant Authority							

☒ Additional inventors are being named on supplemental sheet(s) attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

2-DE

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Rudolf Franciscus Wilhelmus Cornelis		Van Beckhoven	

Inventor: Rudolf Franciscus Wilhelmus Cornelis Van Beckhoven Date: February 6, 1998

Residence: City	State	Country	Citizenship
EK Breda		The Netherlands	The Netherlands

NLX

Post Office Address	Hofwijkstraat 69
Post Office Address	

City	State	Zip	Country	Applicant Authority
4334 EK Breda			The Netherlands	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

3-DE

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Karl-Heinz		Maurer	

Inventor's Signature: Karl-Heinz Maurer Date: February 6, 1998

Residence: City	State	Country	Citizenship
Erkrath		Germany	Germany

DEX

Post Office Address	Dechenstr. 5
Post Office Address	

City	State	Zip	Country	Applicant Authority
40699 Erkrath			Germany	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

4-DE

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Beatrix		Kottwitz	

Inventor's Signature: Beatrix Kottwitz Date: February 6, 1998

Residence: City	State	Country	Citizenship
Duesseldorf		Germany	Germany

DEX

Post Office Address	Urdenbacher Allee 51
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City	State	Zip	Country	Applicant Authority
40593 Duesseldorf			Germany	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

5-DE

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Albrecht		Weiss	

Inventor's Signature: Albrecht Weiss Date: February 6, 1998

Residence: City	State	Country	Citizenship
Langenfeld		Germany	Germany

DEX

Post Office Address	Forellenweg 37
Post Office Address	

City	State	Zip	Country	Applicant Authority
40764 Langenfeld			Germany	

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

6-00

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Pieter	Middle Initial		Family Name	Van Solingen			Suffix e.g. Jr.	
Inventor	Pieter van Solingen					Date	February 6, 1998		
Residence: City	VZ Naaldwijk	State		Country	The Netherlands	Citizenship	The Netherlands		
Post Office Address	Rossini 16								
Post Office Address									
City	2671 VZ Naaldwijk	State		Zip		Country	The Netherlands	Applicant Authority	

NLX

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name		Middle Initial		Family Name				Suffix e.g. Jr.	
Inventor's Signature						Date			
Residence: City		State		Country		Citizenship			
Post Office Address									
Post Office Address									
City		State		Zip		Country		Applicant Authority	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name		Middle Initial		Family Name				Suffix e.g. Jr.	
Inventor's Signature						Date			
Residence: City		State		Country		Citizenship			
Post Office Address									
Post Office Address									
City		State		Zip		Country		Applicant Authority	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name		Middle Initial		Family Name				Suffix e.g. Jr.	
Inventor's Signature						Date			
Residence: City		State		Country		Citizenship			
Post Office Address									
Post Office Address									
City		State		Zip		Country		Applicant Authority	

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PTO/SB/01 (6-95)
Approved for use through: 10/31/98 OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Rev 6/95

U.S. Department of Commerce
Patent and Trademark Office

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing

Attorney Docket Number	H 1920 PCT/US
First Named Inventor	Lenting, Hermanus Bernardus Maria
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:
My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DETERGENTS COMPRISING CELLULASES

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 04/26/1996 as United States Application Number or PCT International Application Number PCT/EP96/01755 and was amended on (MM/DD/YYYY) _____ (if applicable).

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U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
	PCT/EP96/01755	04/26/1996	

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☐ Firm Name ____ Customer Number or label ____

OR

☒ List Attorney(s) and/or agent(s) name and registration number below:

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Wayne C. Jaeschke	21,062	Martin G. Meder	34,674
Real J. Grandmaison	25,981	Glenn E. J. Murphy	33,539
Norvell E. Wisdom, Jr.	30,510		

☐ Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto.

Please direct all correspondence to: ☐ Customer Number or label ____ OR ☒ Fill in correspondence address below

Name	Glenn E. J. Murphy				
Address	Henkel Corporation				
Address	140 Germantown Pike, Suite 150				
City	Plymouth Meeting	State	PA	ZIP	19462
Country	USA	Telephone	610-832-2228	Fax	610-941-6067

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned

Given Name	**Hermanus Bernardus Maria**	Middle Initial		Family Name	**Lenting**	Suffix e.g. Jr.	
Inventor's Signature					Date		
Residence: City	**VT Pijnacker**	State		Country	**The Netherlands**	Citizenship	**The Netherlands**
Post Office Address	**Hunze 5**						
Post Office Address							
City	**2641 VT Pijnacker**	State		Zip		Country	**The Netherlands**
Applicant Authority							

☒ Additional inventors are being named on supplemental sheet(s) attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Rudolf Franciscus Wilhelmus Cornelis		**Van Beckhoven**	

Inventor | Dat

Residence: City	State	Country	Citizenship
EK Breda		**The Netherlands**	**The Netherlands**

Post Office Address: **Hofwijkstraat 69**

Post Office Address:

City	State	Zip	Country	Applicant Authority
4334 EK Breda			**The Netherlands**	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Karl-Heinz		**Maurer**	

Inventor's Signature | Date

Residence: City	State	Country	Citizenship
Erkrath		**Germany**	**Germany**

Post Office Address: **Dechenstr. 5**

Post Office Address:

City	State	Zip	Country	Applicant Authority
40699 Erkrath			**Germany**	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Beatrix		**Kottwitz**	

Inventor's Signature | Date

Residence: City	State	Country	Citizenship
Duesseldorf		**Germany**	**Germany**

Post Office Address: **Urdenbacher Allee 51**

Post Office Address:

City	State	Zip	Country	Applicant Authority
40593 Duesseldorf			**Germany**	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Albrecht		**Weiss**	

Inventor's Signature | Date

Residence: City	State	Country	Citizenship
Langenfeld		**Germany**	**Germany**

Post Office Address: **Forellenweg 37**

Post Office Address:

City	State	Zip	Country	Applicant Authority
40764 Langenfeld			**Germany**	

[X] Additional inventors are being named on supplemental sheet(s) attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	**Pieter**	Middle Initial		Family Name	**Van Solingen**	Suffix e.g. Jr.	
Inventor						Date	
Residence: City	**VZ Naaldwijk**	State		Country	**The Netherlands**	Citizenship	**The Netherlands**
Post Office Address	**Rossini 16**						
Post Office Address							
City	**2671 VZ Naaldwijk**	State		Zip		Country	**The Netherlands**
Applicant Authority							

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
Applicant Authority							

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Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
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City		State		Zip		Country	
Applicant Authority							

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Given Name		Middle Initial		Family Name		Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
Applicant Authority							

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U.S. Application No. (If known see CFR1.30)	INTERNATIONAL APPLICATION NO. **PCT/EP96/01755**	ATTORNEY'S DOCKET NUMBER **H 1920 PCT/US**

17. ■ The following fees are submitted:

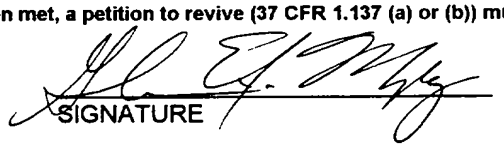
	CALCULATIONS ONLY	PTO USE
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO..... **$930.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482) **$720.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37CFR 1.445(a)(2))..... **$790.00**		
Neither international preliminary examination fee (37CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$1070.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... **$98.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930	00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date 37 (CFR 1.492(e)).	$ 0	00

Claims	Number filed	Number Extra	Rate		
Total Claims	1 - 20 =	0	0 X $22.00	$ 0	00
Independent Claims	1 - 3 =	0	0 X $82.00	$ 0	00
Multiple dependent claims (s)(if applicable)	0		+ $270.00	$ 0	00
TOTAL OF ABOVE CALCULATIONS =				$ 930	00
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0	00
SUBTOTAL =				$ 930	00
Processing fee of **$130.00** for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37CFR 1.492(f)). +				$ 0	00
TOTAL NATIONAL FEE =				$ 930	00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 0	00
TOTAL FEES ENCLOSED =				$ 930	00
				Amount to be: refunded	$
				charged	$930.00

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.
b. ■ Please charge my Deposit Account No. 01-1250 in the amount of $930.00 to cover the above fees. A triplicate copy of this sheet is enclosed. Order No. 97-1230.
c. ■ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-1250. A triplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Henkel Corporation, Law Dept.
140 Germantown Pike, Suite 150
Plymouth Meeting, PA 19462



SIGNATURE

Glenn E. J. Murphy
NAME ATTORNEY FOR APPLICANT
33,539
REGISTRATION NUMBER

Form PTO 1390 (REV 5-93)